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(71) Applicant *(for all designated States except US)*: GLAXO GROUP LIMITED [GB/GB]; Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: SMITH, Paul, William [GB/GB]; Glaxo Research and Development Limited, Greenford Road, Greenford, Middlesex UB6 0HE (GB).

(74) Agents: QUILLIN, Helen, Kaye et al.; Glaxo Holdings plc, Glaxo House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB).

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(54) Title: DERIVATIVES OF 2-DEOXY-2,3-DEHYDRO-N-ACETYLNEURAMINIC ACID (DANA)

(57) Abstract

(4S, 5R, 6R)-4-Guanidino-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxypropyl-5,6-dihydro-4H-pyran-3-carboxylic acid and its physiologically acceptable derivatives and solvates are inhibitors of viral neuraminidase.

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# DERIVATIVES OF 2-DEOXY-2,3-DEHYDRO-N-ACETYLNEURAMINIC ACID (DANA)

This invention relates to a chemical compound and to its use in medicine. In particular the invention concerns a novel α-D-neuraminic acid derivative, methods for its preparation, pharmaceutical formulations containing it and its use as an antiviral agent.

Enzymes with the ability to cleave N-acetyl neuraminic acid (NANA), also known as sialic acid, from other sugars are present in many micro-organisms. These include bacteria such as Vibrio cholerae, Clostridium perfringens, Streptococcus pneumoniae, and Arthrobacter sialophilus, and viruses such as influenza virus, parainfluenza virus, mumps virus, Newcastle disease virus, fowl plague virus, and Sendai virus. Most of these viruses are of the orthomyxovirus or paramyxovirus groups, and carry a neuraminidase activity on the surface of the virus particles.

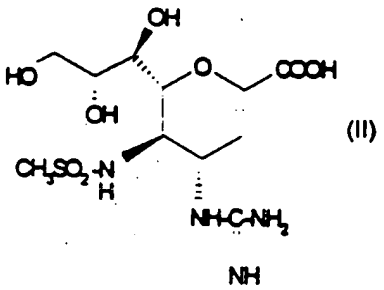
Many of the neuraminidase-possessing organisms are major pathogens of man and/or animals, and some, such as influenza virus, Newcastle disease virus, and fowl plague virus, cause diseases of enormous economic importance.

It has long been thought that inhibitors of neuraminidase activity might prevent infection by neuraminidase-bearing viruses. Most of the known neuraminidase inhibitors are analogues of neuraminic acid, such as 2-deoxy-2,3-didehydro-N-acetylneuraminic acid (DANA) and its derivatives. See, e.g., Meindl et al., Virology 1974 58 457-63. The most active of these is 2-deoxy-2,3-dehydro-N-trifluoroacetyl-neuraminic acid (FANA), which inhibits multi-cycle replication of influenza and parainfluenza viruses in vitro. See Palese et al., Virology 1974 59 490-498.

International Application Publication No. WO91/16320 describes a number of analogues of DANA, active both in vitro and in vivo against viral neuraminidase and useful in the treatment of influenza.

W have now found a novel compound which is active against the influenza virus.

The present invention accordingly provides (4S, 5R, 6R)-4-guanidino-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxy-propyl- 5,6-dihydro-4H-pyran-3-carboxylic acid of formula (II)



(II)

and its physiologically acceptable derivatives and solvates (e.g. hydrates)

By "a pharmaceutically acceptable derivative" is meant any pharmaceutically acceptable salt, ester, or salt of such ester, of the compound of formula (II) or any other compound which, upon administration to the recipient, is capable of providing (directly or indirectly) the compound of formula (II) or an antivirally active metabolite or residue thereof.

It will be appreciated by those skilled in the art that the compound of formula (II) may be modified to provide pharmaceutically acceptable derivatives thereof at any of the functional groups in the compound of formula (II). Of particular interest as such derivatives are compounds modified at the carboxyl function, hydroxyl functions or at amino groups. Thus compound of interest include alkyl (such as methyl, ethyl or propyl e.g. isopropyl) or aryl (e.g. phenyl, benzoyl) esters and acetyl esters of the compound of formula (II).

It will be appreciated by those skilled in the art that the pharmaceutically acceptable derivatives of the compound of formula (II) may be derivatised at more than one position.

Pharmaceutically acceptable salts of the compound of formula (II) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene- p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, while not in themselves pharmaceutically acceptable may be useful in the preparation of salts useful as intermediates in obtaining the compound of the invention and its pharmaceutically acceptable acid addition salts.

Salts derived from appropriate bases include alkali metal (e.g. sodium), alkaline earth metal (e.g. magnesium), ammonium and $NR_4^+$ (where R is $C_{1-4}$alkyl) salts.

References hereinafter to the compound of the invention include the compound of formula (II) and pharmaceutically acceptable derivatives thereof.

The compound of formula (II) possesses antiviral activity. In particular this compound is an inhibitor of viral neuraminidase of orthomyxoviruses and paramyxoviruses in particular neuraminidase, for example the viral neuraminidase of influenza A and B, parainfluenza, mumps and Newcastle disease.

There is thus provided in a further aspect of the invention the compound of formula (II) or a pharmaceutically acceptable derivative thereof for use as an active therapeutic agent in particular as an antiviral agent for example in the treatment of orthomyxovirus and paramyxovirus infections.

In a further or alternative aspect there is provided a method for the treatment of a viral infection, for example orthomyxovirus and paramyxovirus infections in a mammal including man comprising administration of an effective amount of the compound of formula (II) or a pharmaceutically acceptable derivative thereof.

There is also provided in a further or alternative aspect use of the compound of the invention for the manufacture of a medicament for the treatment of a viral infection.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established infections or symptoms.

It will be further appreciated that the amount of the compound of the invention required for use in treatment will vary with the route of administration, the nature of the condition being treated and the age and condition of the patient and will ultimately be at the discretion of the attendant physician or veterinarian. In general however a suitable dose will be in the range of from about 0.1 to 750mg/kg of bodyweight per day, preferably in the range of 0.5 to 60 mg/kg/day, most preferably in the range of 1 to 20mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until virus is no longer present in the respiratory tract. However the compound is also effective when given post-infection, for example after the appearance of established symptoms.

Suitably treatment is given 1-4 times daily and continued for 3-7, e.g. 5 days post infection.

The desired dose may be presented in a single dose or as divided doses administered at appropriate intervals, for example as two, three, four or more sub-doses per day.

The compound is conveniently administered in unit dosage form for example containing 10 to 1500mg, conveniently 20 to 1000mg, most conveniently 50 to 700mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, the compound of the invention may be administered as the raw chemical it is preferable to present the active ingredient as a pharmaceutical formulation.

The invention thus further provides a pharmaceutical formulation comprising the compound of formula (II) or a pharmaceutically acceptable derivative thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be 'acceptable' in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration or in a form suitable for administration to the respiratory tract (including the nasal passages) for example by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing into association the active compound with liquid carriers or finely divided solid carriers or both and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution, a suspension or as an emulsion. The active ingredient may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles (which may include edible oils), or preservatives.

The compound according to the invention may also be formulated for parenteral administration (e.g. by injection, for example bolus injection or continuous

infusion) and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilising and/or dispersing agents. Alternatively, the active ingredient may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, e.g. sterile, pyrogen-free water, before use.

For topical administration to the epidermis the compound according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising active ingredient in a flavoured base, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert base such as gelatin and glycerin or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the active compound with the softened or melted carrier(s) followed by chilling and shaping in moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For administration to the respiratory tract (including intranasal administration) according to the method of the invention the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for administration to the respiratory tract.

Thus in general the compounds may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous for example prepared from water alone (for example sterile or pyrogen-free water) or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol, polyethlene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (e.g. Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the patient administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant such as a chlorofluorocarbon (CFC) for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluroroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also

contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the compounds may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form for example in capsules or cartridges of e.g. gelatin or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the compound will generally have a small particle size for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired, formulations adapted to give sustained release of the active ingredient may be employed.

The compound of the invention may also be used in combination with other therapeutic agents, for example other anti-infective agents. In particular the compound of the invention may be employed with other antiviral agents. The invention thus provides in a further aspect a combination comprising the compound of formula (II) or a pharmaceutically acceptable derivative thereof together with another therapeutically active agent, in particular an antiviral agent.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations comprising a combination as defined above together with a pharmaceutically acceptable carrier therefor comprise a further aspect of the invention.

Suitable therapeutic agents for use in such combinations include other anti-infective agents, in particular anti-bacterial and anti-viral agents such as those used to treat respiratory infections. For example, other compounds effective

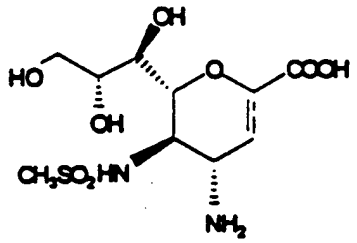
against influenza viruses, such as amantadine, rimantadine and ribavirin, may be included in such combinations.

The individual components of such combinations may be administered either sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the compound of the invention is used with a second therapeutic agent active against the same virus the dose of each compound may either be the same as or differ from that employed when each compound is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

The compound of formula (II) and its pharmaceutically acceptable derivatives may be prepared by any method known in the art for the preparation of compounds of analogous structure. In particular the compounds of formula (II) may be prepared by the methods described below.

The compound of formula (II) may be prepared from the compound of formula (III)

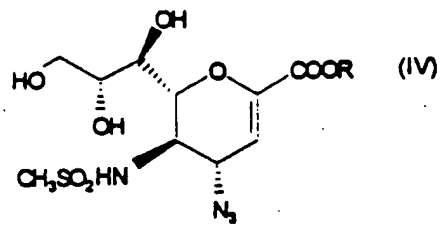



(III)

by treatment with a reagent suitable to introduce the guanidino function.

Reagents suitable to introduce the guanidino function include S-methylisourea and aminoiminomethanesulphonic acid in the presence of a base such as an alkali metal carbonate, for example potassium carbonate.

The compound of formula (III) may be prepared from an azide of formula (IV)

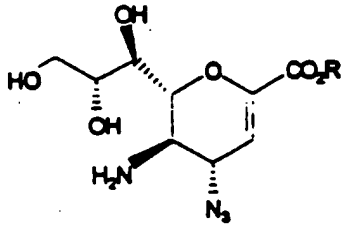



(IV)

wherein R represents alkyl, such as, for example, methyl, by reduction.

The reduction is conveniently effected using triphenylphosphine in a suitable solvent such as an ether, for example tetrahydrofuran, in the presence of a base. Suitable bases include tertiary amines such as, for example, triethylamine.

Azides of formula (IV) may be prepared from compounds of formula (V)

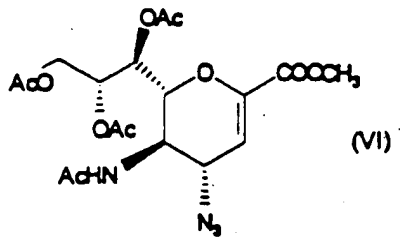



(V)

by treatment with a methanesulphonyl halide, such as methanesulphonyl chloride, in the presence of a base, such as a tertiary amine, for example, pyridine..

The reaction is conveniently effected in a suitable organic solvent, such as a halogenated hydrocarbon, for example, dichloromethane, preferably at low temperature, such as about 0°C.

Compounds of formula (V) may be prepared by conventional methods from a suitably protected analogue, such as the compound of formula (VI) (4-azido-Neu5, 7, 8, 9-$Ac_4$5Boc2en1Me)




(VI)

The preparation of the compound of formula (VI) is described in WO91/16320.

As will be appreciated by those skilled in the art it may be necessary or desirable at any stage in the above described processes to protect one or more sensitive groups in the molecule to prevent undesirable side reactions; the protecting group may be removed at any convenient subsequent stage in the reaction sequence.

The protecting groups used in the preparation of compounds of formula (II) may be used in conventional manner. See for example 'Protective Groups in Organic Chemistry' Ed. J. F. W. McOmie (Plenum Press 1973) or 'Protective Groups in Organic Synthesis' by T. W . Greene and P.G. M. Wuts (John Wiley and Sons 1991).

Conventional amino protecting groups may include for example aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups; and acyl groups such as N-benzyloxycarbonyl or t-butoxycarbonyl.

Hydroxy groups may be protected, for example, by aralkyl groups, such as benzyl, diphenylmethyl or triphenylmethyl groups, acyl groups, such as acetyl, silicon protecting groups, such as trimethylsilyl groups, or as tetrahydropyran derivatives.

Removal of any protecting groups present may be achieved by conventional procedures. Thus an aralkyl group such as benzyl, may be cleaved by hydrogenolysis in the presence of a catalyst (e.g. palladium on charcoal); an acyl group such as N-benzyloxycarbonyl may be removed by hydrolysis with, for example, hydrogen bromide in acetic acid or by reduction, for example by catalytic hydrogenation; silicon protecting groups may be removed, for example,

by treatment with fluorid ion; tetrahydropyran groups may be cleaved by hydrolysis under acidic conditions

Where it is desired to isolate the compound of the invention as a salt, for example as an acid addition salt, this may be achieved by treating the free base of general formula (I) with an appropriate acid, preferably with an equivalent amount, or with creatinine sulphate in a suitable solvent (e.g. aqueous ethanol).

The present invention is further described by the following examples which are for illustrative purposes only and should not be construed as a limitation of the invention.

Example 1

(4S, 5R, 6R)-4-Guanidino-5-methanesulphonylamino-6-[(1S, 2R)-1,2,3-trihydroxy-propyl-5,6-dihydro-4H-pyran-3-carboxylic acid bis (trifluoroacetate).

To (4S,5R,6R)-5-(acetoxy-amino)-4azido-6-[(1S,2R)-1,2,3-triacetoxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester (10g) in 1,4-dioxan (100ml) was added di-tert-butyl dicarbonate (9.55g) and 4-dimethylamino pyridine (500mg). After 72 hours the solvent volume was reduced by approximately two thirds and a second aliquot of di-tert-butyl dicarbonate (3g) added. Seventeen hours later, a third batch of di-tert-butyl dicarbonate (3g) was added and the reaction heated at 80°C for 2 hours. The product was isolated by removing the dioxan *in vacuo* and chromatographing the resulting oil on flash silica gel (Merck 9385) with ethyl acetate/cyclohexane (2:3) to yield (4S,5R,6R)-5-[acetoxy-(t-butoxycarbonyl)-amino]-4- azido-6-[(1S,2R)-1,2,3-triacetoxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester as an orange oil (10.46g). $H^1$ nmr ($DMSOd_6$) δ 5.99 (br.s,1H), 5.27-5.15 (m,2H), 4.95-4.65(m,3H), 4.5(m,1H), 4.15-4.02(m,1H), 3.75 (s,3H), 2.35 (s,3H), 1.99 (s,9H), 1.55 (s,9H); MS M/Z 574 $MNH^+_4$, 474 $MNH^+_4$ - BOC

(4S,5R,6R)-5-[Acetoxy-(t-butoxycarbonyl)- amino]-4-azido-6-[(1S,2R)-1,2,3-triacetoxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester (5.05g) and sodium methoxide (30% w.v solution 0.26mL) were stirred under nitrogen in methanol (30ml). After 60 minutes more methanol was added to dissipate the forming precipitate. After 17 hours the solvent was removed *in vacuo* to give a

brown solid which was further stirred with both diethyl ether (100ml) and water (100ml) for 15 minutes. The insoluble white precipitate was collected by vacuum filtration and dried overnight *in vacuo* (60°C) to give (4S,5R,6R)-4-azido-5-(t-butoxycarbonyl-amino)-6-[(1S,2R)-1,2,3-trihydroxy-propyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester as white crystals (2.48g). $^1$H nmr (DMSO$d_6$) δ 7.23 (d,9Hz,1H), 5.79(d,2Hz,1H), 4.65 (d,5Hz,1H), 4.49-4.34(m,3H), 4.15 (d,11Hz,1H), 3.75-3.54(m,3H), 3.52-3.37(m,2H), 3.72(s,3H) 1.40(s,9H); Calculated for $C_{15}H_{24}N_4O_8$; C,46.39; H,6.23; N,14.43; found C,46.08; H,6.07 N,14.44.

A stirred solution of (4S,5R,6R)-4-azido-5-(t-butoxycarbonyl-amino)-6-[(1S,2R)-1,2,3-trihydroxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester (50mg ) in acetic anhydride (500μL) and pyridine (500μL) was stirred for 3 hours. Excess acetic anhydride was destroyed with ethanol (2ml) and the solution reduced to an oil by evaporation *in vacuo*. This oil was taken up in ethyl acetate (30ml) and washed with 2N hydrochloric acid (20ml), dried ($MgSO_4$) and evaporated *in vacuo* to a clear oil. To remove final traces of acetic acid the oil was co-evaporated with toluene (2x2ml) and finally ether (5ml) to give (4S,5R,6R)-4-azido-5-(t-butoxycarbonyl-amino)-6-[(1S,2R)-1,2,3-triacetoxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester as a white foam (52mg).$^1$H nmr (DMSO$d_6$) δ 7.18 (d,10Hz,1H), 5.83 (d,2Hz,1H), 5.36 (dd,6,1Hz,1H), 5.23 (m,1H), 4.45 (dd,12,2Hz, 1H,), 4.32 (dd,9,2Hz,1H), 4.22 (dd, 1,10Hz ,1H), 4.07 (dd,7,12Hz,1H), 3.74 (m,1H), 3.72(s,3H), 2.03 (s,3H), 1.98 (s,6H), 1.37(s,9H); IR(KBr) 2098, 1745, 1721; MS M/Z=532 $MNH_4^+$.

A stirred solution of (4S,5R,6R)-4-azido-5-(t-butoxycarbonyl-amino)-6-[(1S,2R)-1,2,3-triacetoxy-propyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester (3g) in 1,4 dioxan (5ml) was treated with 4M hydrochloric acid/dioxan (10ml). After 3 hours the solvent was removed to yield (4S,5R,6R)-5-amino-4-azido-6-[(1S,2R)-1,2,3 triacetoxy-propyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester hydrochloride salt as a beige foam (2.6g). $^1$H nmr (DMSO$d_6$) δ 8.74 (brs,3H), 6.29(d,4Hz,1H), 5.45-5.33(m,2H), 4.68(dd,3,7Hz,1H), 4.63(t,3Hz,1H), 4.39(dd,12,2Hz,1H), 4.20(dd,12,7Hz,1H) 3.75(m, 1H) 3.74(s,3H), 2.07 (s,3H), 2.03(s,3H), 2.01(s,3H).

A solution of (4S,5R,6R)-5-amino-4-azido-6-[(1S,2R)-1,2,3-triacetoxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester hydrochloride (300mg) in anhydrous dichloromethane (4ml) was treated, under nitrogen, with dry pyridine (4ml) and methanesulphonyl chloride (80μL) at 0°C. After 2 hours the solution was partitioned between 2N hydrochloric acid (50ml) and ethyl acetate (50ml). The ethyl acetate layer was separated, dried ($MgSO_4$) and evaporated to a yellow foam. Column chromatography gave (4S,5R,6R)-4- azido-5-methanesulphonylamino-6-[(1S,2R)-1,2,3- triacetoxy-propyl]-5,6-dihydro-4H-pyran-2 carboxylic acid methyl ester as a white foam (225mg). $^1H$ nmr ($DMSOd_6$) δ 7.59 (d,8Hz,1H), 6.04 (d,2Hz,1H), 5.42 (m,1H), 5.27(m,1H), 4.45(m,1H), 4.39(m,1H) 4.25(dd,2,10$H_z$,1H), 4.09(dd,8,10Hz, 1H), 3.45 (m,1H), 3.73 (s,3H), 3.05(s,3H), 2.04-2.02(m,9H); ms M/Z=510 $MNH_4^+$

A solution of (4S,5R,6R)-4-azido-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-triacetoxy-propyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester (160mg) in dry methanol (3ml) was treated with a 30% w/v solution of sodium methoxide (10μL). The reaction was stirred under nitrogen for 17 hours before removal of the solvent *in vacuo* and chromatography of the residual gum to give (4S,5R,6R)4-azido-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxy-propyl 5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester as a white powder (85mg).$^1H$ nmr ($DMSOd_6$) δ 7.70(br.s,1H), 5.87(d,2Hz, 1H), 4.70 (d,3Hz,1H), 4.65 (d,7Hz,1H), 4.45 (t,7Hz,1H), 4.25 (dd,2,10Hz, 1H), 4.11(d,10Hz,1H), 3.72-3.40 (m,5H), 3.73(s,3H), 3.06(s,3H); ms M/Z=384 $MNH_4^+$; Calculated for $C_{11}H_{18}N_4O_8S.0.18CHCl_3$: C,34.62; H,4.72; N,14.45; S, 8.27; found C,34.63; H,4.72; N,14.27; S,8.30.

A solution of (4S,5R,6R)-4-azido-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxypropyl]-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester, (75mg) in 1:1 water : tetrahydrofuran (3ml) was treated with triphenylphosphine (107mg). After 35 hours, triethylamine (150μL) was added and the reaction stirred at room temperature for 2 days before partitioning between water (20ml) and ethyl acetate (20ml). The aqueous layer was collected and freeze-dried to give a yellow solid which was purified by preparative h.p.l.c. to give (4S,5R,6R)-4-amino-5-methanesulfonylamino-6-[(1S,2R)-1,2,3-trihydroxy-propyl]-5,6-dihydro-4H-pyran-2-carboxylic acid as a white solid (56mg). $^1H$ nmr ($D_2O$) δ

5.78 (d,2Hz, 1H,),4.40 (d,10Hz,1H), 4.19 (dd,10,2Hz,1H), 4.05-3.83 (m,4H), 3.73(dd,12,6Hz, 1H), 3.23(s,3H); ms M/Z=327 $MNH_4^+$ for $C_{10}H_{19}N_2O_8S$=327.086213.

(4S,5R,6R)-4-Amino-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxy-propyl]-5,6-dihydro-4H-pyran carboxylic acid (88mg) in water (4ml) was treated with potassium carbonate (75mg) to form a suspension. Over 1 hour an intimate mixture of potassium carbonate (112mg) and aminoiminomethane sulphonic acid (AIMSA) (100mg) was added. One day later, a second identical mixture (AIMSA/$K_2CO_3$) was added over 7 hours and likewise on the third day. The reaction was left to stir for 68 hours before diluting with water and warming gently to give a solution. This solution was eluted through a DOWEX 50W-X8(H) first with water (until pH7 returned) and then with 0.6M triethylamine in water to give a crude grey solid. This solid was subjected to preparative hplc to give three major peaks. The fraction which gave a positive SAKAGUCHI test and was freeze dried and repurified by preparative hplc to give the *title compound* as a white solid (18mg). $^1H$ nmr ($D_2O$) δ 5.70 (d,2Hz,1H), 4.32(d,10Hz,1H), 4.22(d,10Hz,1H), 3.86-3.64 (m,4H), 3.57 (dd,11,5$H_z$,1H), 3.03(s,3H); ms measured 369.107803 $C_{11}H_{21}N_4O_8S$ requires 369.108011.

## CLAIMS

1. (4S, 5R, 6R)-4-Guanidino-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxy-propyl-5,6-dihydro-4H-pyran-3-carboxylic acid and physiologically acceptable derivatives and solvates thereof.

2. A compound as claimed in Claim 1 for use in therapy.

3. The use of a compound as claimed in Claim 1 for the manufacture of a medicament for the treatment of a viral infection.

4. A method of treatment of a viral infection in a mammal which method comprises administration to said mammal of an effective amount of a compound as claimed in Claim 1.

5. A pharmaceutical composition comprising a compound as claimed in Claim 1 and a pharmaceutically acceptable carrier therefor.

6. A pharmaceutical composition as claimed in Claim 5 adapted for administration to the respiratory tract.

7. A process for the preparation of a compound as claimed in Claim 1, which process comprises reaction of (4S,5R,6R)-4-amino-5-methanesulphonylamino-6-[(1S,2R)-1,2,3-trihydroxypropyl]-5,6-dihydro-4H-pyran carboxylic acid, or a protected derivative thereof, with a guanidinating agent.

# INTERNATIONAL SEARCH REPORT

Int. onal Application No
PCT/EP 95/00040

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D309/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO,A,91 16320 (BIOTA) 31 October 1991<br>cited in the application<br>see claims | 1-5 |

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 29 March 1995 | 11. 04. 95 |

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte rnal Application No
PCT/EP 95/00040

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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